

REMARKS

The above amendatory action is taken in response to the Official Action of May 4, 2001 and satisfies the requirements of 37 CFR 1.821 - 1.825. A computer readable form copy of the Sequence Listing and the requisite statements are submitted herewith. Also submitted herewith is a marked-up copy of the amended paragraphs of the specification to locate the amendments.

~~Respectfully submitted,~~

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RNA levels of IFN α and IFN β were determined using a quantitative polymerase chain reaction reverse transcription (RT-PCR) method using a thermocycler (Perkin-Elmer Gene Amp PCR system 2400). Prior to reverse transcription 2 μ g of total RNA (from both the liver and PBMC) were treated with 1 unit of deoxyribonuclease (DNase I amplification grade, Gibco-BRL, Gaithersburg, MD, USA) to eliminate possible contaminating DNA. The presence of traces of DNA was checked by including control reactions without reverse transcription. This step is required because of the absence of introns in IFN α and IFN β genes (18), which made it impossible for us to distinguish the product of PCR from the RNA or possible contaminating DNA. All the controls performed without reverse transcription were negative, indicating the absence of contaminating DNA. Total RNA was transcribed (60 minutes at 37°C) with 400 units of M-MuLV reverse transcriptase (Gibco- BRL, Gaithersburg, MD, USA) in a final volume of 40 μ l of 5 x saline solution (250 mM Tris-HCl pH 8.3, 375 mM KCl, 15 mM MgCl₂), supplemented with 5 mM DTT, 0.5 mM triphosphate dioxyribonucleotides (Boehringer Mannheim, Mannheim, Germany), 48 units of RNAsas inhibitor (Promega Corporation, MD, US) and 400 ng of random hexamers (Boehringer Mannheim, Mannheim, Germany). After denaturing the reverse transcriptase (95°C, 1 minute) and rapidly cooling over ice, a 10 μ l aliquot (0.5 μ g) of the cDNA was used to amplify the IFN α and IFN β by PCR in 50 μ l of 10 x PCR buffer (160 mM (NH₄)SO₄, 670 mM Tris-HCl pH 8.8, 0.1% Tween 20) supplemented with the direction and antidiirection primers (40 ng of each one for IFN α and 60 ng for IFN β), 1.2 mM MgCl₂ and 2 units of Biotaq™ DNA polymerase (Bioline, London, LTK). Control reactions without RNA were performed in all the experiments. As an internal control for each sample a

fragment of β -actin cDNA was amplified using a 10 μ l aliquot of the cDNA obtained previously. The IFN α was amplified by performing 30 or 33 cycles (PBMC or liver respectively) (94°C, 60°C and 72°C during 20, 15 and 30 seconds for each step respectively), the INF β was amplified by performing 30 or 35 cycles (PBMC or liver respectively) (94°C, 58°C and 72°C for 20, 15 and 30 seconds for each step respectively) and β -actin was amplified by reacting 18 or 25 cycles (PBMC or liver respectively) (94°C, 55°C and 72°C for 20, 15 and 30 seconds for each step respectively), protocols which avoid interference with the PCR reaction saturation stage. The oligonucleotides (5'-3') d(TCCATGAGATGATCCAGCAG) (SEQ ID NO:2) and d(ATTTCTGCTCTGACAACCTCCC) (SEQ ID NO:3) were used as direction and antidirection primers respectively to amplify a fragment of 274 pairs of bases located between nucleotides 240-514 in the human IFN α gene (19). These oligonucleotides are direction primers designed to amplify all the subtypes of IFN α . The oligonucleotides D(TCTAGCACTGGCTGGAATGAG) (SEQ ID NO:4) and d(GTTTCGGAGGTAACCTGTAAG) (SEQ ID NO:5) were the primers used to amplify a fragment of 276 base pairs located between nucleotides 349-625 of cDNA of human IFN β (20). d(TCTACAATGAGCTGCGTGTG) (SEQ ID NO:6) and d(GGTGAGGATCTTCATGAGGT) (SEQ ID NO:7) were the primers used to amplify a fragment of 314 base pairs (nucleotides 1319-2079) of the β -actin gene (21).

Please rewrite the paragraph bridging pages 12 - 13 as follows:

The presence of C virus RNA in serum was determined using the RT-PCR technique (14, 22), using 2 pairs of specific primers for the non-coding 5' region of the C virus genome. The C virus RNA was quantified using the competitive PCR technique previously described by

ourselves (22). The viral genotype was determined using Viazov's method (23) as already described previously (22, 24). The test 5'G(A,G)(R)CCGTCTTGGGGCC(A,C)(M)AAATGAT (SEQ ID NO:8) was used to determine genotype 4.